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### Citation for published version:

Mcneil, M, Gerber, PF, Thomson, J, Williamson, S & Opriessnig, T 2017, 'Serotypes and Spa types of *Erysipelothrix rhusiopathiae* isolates from British pigs (1987 to 2015)', *The Veterinary Journal*, vol. 225, pp. 13-15. <https://doi.org/10.1016/j.tvjl.2017.04.012>

### Digital Object Identifier (DOI):

[10.1016/j.tvjl.2017.04.012](https://doi.org/10.1016/j.tvjl.2017.04.012)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Peer reviewed version

### Published In:

The Veterinary Journal

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## Accepted Manuscript

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PII: S1090-0233(17)30090-4  
DOI: <http://dx.doi.org/doi:10.1016/j.tvjl.2017.04.012>  
Reference: YTVJL 4986

To appear in:

Received date: 20-10-2016  
Revised date: 8-3-2017  
Accepted date: 21-4-2017

Please cite this article as: Mark McNeil, Priscilla F. Gerber, Jill Thomson, Susanna Williamson, Tanja Opriessnig, Serotypes and Spa types of *Erysipelothrix rhusiopathiae* isolates from British pigs (1987 to 2015) (2010), <http://dx.doi.org/10.1016/j.tvjl.2017.04.012>

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**Short communication****Serotypes and Spa types of *Erysipelothrix rhusiopathiae* isolates from British pigs (1987 to 2015)**

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**Highlights**

- *Erysipelothrix* spp. are important pathogens in pig production.
- British *Erysipelothrix rhusiopathiae* isolates from 1987 to 2015 were characterised.
- Serotype 2 was the most common serotype identified.

**Abstract**

*Erysipelothrix* spp. cause a range of clinical signs in pigs and at least 28 different *Erysipelothrix* spp. serotypes have been identified. In this study, 128 isolates of *Erysipelothrix* spp. from pigs in Great Britain from 1987 to 2015 were characterised by serotyping and multiplex real time PCR assays targeting the surface protective antigen (Spa) and the main genotypes (*Erysipelothrix rhusiopathiae*, *Erysipelothrix tonsillarum* and *Erysipelothrix* spp. strain 2). All 128 British isolates were characterised as *E. rhusiopathiae* and were classified as serotypes 1a ( $n = 21$ ), 1b ( $n = 17$ ), 2 ( $n = 75$ ), 5 ( $n = 2$ ), 9 ( $n = 2$ ), 10 ( $n = 2$ ), 11 ( $n = 4$ ) and 15 ( $n = 1$ ), while four isolates were untypeable. All isolates were positive for the spa A gene. Serotypes 1a, 1b and 2 constituted 88.3% of the isolates; current serotype 2 based vaccines should protect against these isolates.

**Keywords:** Porcine; *Erysipelothrix* spp.; Serotype; Characterisation

*Erysipelothrix* spp. are Gram positive bacilli that cause disease in a wide range of wild and domestic species, including birds, mammals, reptiles, fish, arthropods and human beings (Opriessnig and Wood, 2012). In pigs, the main *Erysipelothrix* spp. host, the bacterium can cause significant economic losses due to outbreaks of acute septicaemia, with or without cutaneous lesions, abortions in breeding sows or chronic infection causing endocarditis and arthritis. There are four *Erysipelothrix* spp. and 28 different serotypes; *Erysipelothrix rhusiopathiae* (serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, 23 and N), *Erysipelothrix tonsillarum* (serotypes 3, 7, 10, 14, 20, 22, 24, 25 and 26), *Erysipelothrix* spp. strain 1 (serotype 13) and *Erysipelothrix* spp. strain 2 (serotype 18). *E. rhusiopathiae* serotypes 1 and 2 are frequently isolated from clinically affected pigs and have the highest prevalence and economic importance (Opriessnig and Wood, 2012).

In recent years, there has been an increase in the number of *Erysipelothrix* spp. outbreaks in Europe, the USA and Japan (Bender et al., 2011; To et al., 2012), and there is anecdotal evidence of increased outbreaks of erysipelas and apparent vaccine failure in Great Britain. The aim of this study was to characterise *Erysipelothrix* spp. isolates obtained during the last four decades from British pigs. A total of 128 *Erysipelothrix* spp. isolates from 120 cases submitted from 14 geographic regions in Great Britain were supplied by the Scottish Agricultural College (SAC) Veterinary Services, Scotland, and the Animal and Plant Health Agency (APHA), England and Wales. The isolates originated from samples of heart, spleen, lung, kidney and joint swabs of farmed British pigs with clinical signs consistent with erysipelas and were collected from 1987 to 2015. Clinical signs included arthritis, skin lesions and sudden death. Eight of 128 (6.3%) isolates were collected during 1987-1989, 41/128 (32%) were collected during 1990-1999, 38/128 (29.7%) were collected during 2000-2009 and 41/128 (32%) were collected during 2010-2015.

All isolates in the current study set were initially identified as *E. rhusiopathiae* by multiplex PCR and the genotypes were determined (Bender et al., 2011). Serotyping was performed using an

agar gel immunodiffusion assay based on rabbit antisera generated against serotypes 1-26 (Bender et al., 2011). Amongst all 128 *Erysipelothrix* spp. isolates, eight serotypes were identified and included 1a, 1b, 2, 5, 9, 10, 11 and 15 (Table 1). The most prevalent serotype was 2, followed by serotypes 1a and 1b; these serotypes constituted 88.3% of isolates.

Serotype 1 and 2 strains are commonly used in live vaccines or inactivated bacterins to control disease due to *E. rhusiopathiae* infection (Opriessnig and Wood, 2012). In the United Kingdom, the only *E. rhusiopathiae* vaccine currently available is an inactivated bacterin based on serotype 2. Cross protection amongst serotypes 1a, 1b and 2 has been demonstrated (Opriessnig and Wood, 2012).

The remaining 11.7% of the isolates included serotypes 5, 9, 10, 11 and 15 and four untypeable isolates, which did not react with any of the antisera used under the study conditions. Vaccination status and clinical signs from serotypes 1a, 1b and 2 are summarised in Table 2. The distribution of the different serotypes over time is summarised in Table 1.

Surface protective antigens (Spa) of *Erysipelothrix* spp. have been associated with virulence and the induction of an antibody response during infection (Ingebritson et al., 2010). Four Spa types (SpaA, SpaB1, SpaB2 and SpaC) have been identified. SpaA is associated with serotypes 1a, 1b, 2, 5, 9, 12, 15, 16, 17, 23 and N, SpaB1 is associated with serotypes 4, 6, 8, 19 and 21, SpaB2 is associated with serotype 11 and SpaC is associated with serotype 18. *E. tonsillarum* contains no known Spa proteins (Makino et al., 2000). Although most *E. rhusiopathiae* strains have been described as possessing a single Spa type, strains isolated from fish may carry more than one Spa type (Ingebritson et al., 2010).

In this study, the Spa type was confirmed by a multiplex real-time PCR assay capable of

detecting and differentiating SpaA, SpaB1, SpaB2 and SpaC (Shen et al., 2010). Among the 128 *E. rhusiopathiae* isolates, 127 isolates were positive for SpaA. Of the four untypeable isolates by serotyping, three were positive for the SpaA gene. All four serotype 11 isolates were positive for SpaA, whereas previous serotype 11 isolates contain SpaB2 (Shen et al., 2010). Furthermore, both serotype 10 isolates from samples originating from different farms in 2006 and 2007, each propagated from a single bacterial colony for further characterisation, were PCR positive for SpaA and for *E. rhusiopathiae*. From previous studies using serotype 10 reference strains and limited numbers of field strains, this serotype clusters normally with *E. tonsillarum* isolates and does not contain any known Spa genes (Shen et al., 2010). The present findings indicate that genetic differences among geographically distinct *Erysipelothrix* spp. isolates may exist. To account for this, more than one assay targeting different genetic regions/bacterial properties of *Erysipelothrix* spp. may need to be used for characterisation.

The main serotype identified in British pigs over the last four decades was *E. rhusiopathiae* serotype 2. An Australian study which used isolates collected during 1995-1998 identified serotype 1a on 11 farms, 1b on 6 farms and serotype 2 on 16 farms (Eamens et al., 2006). Among *Erysipelothrix* spp. isolates recovered from 2008-2011 outbreaks in Japan, serotype 1a was the most prevalent (79/83 isolates; 95.2%), followed by serotype 2 (1/83 isolates; 1.2%) (To et al., 2012). In a study investigating serotypes in the USA from 1999 to 2001, 35/44 (79.6%) isolates were serotype 1a, 6/44 (13.6%) isolates were serotype 1b and 3/44 (6.8%) isolates were serotype 2 (Opriessnig et al., 2004). Eighteen serotypes of *Erysipelothrix* spp. were identified among 151 isolates from Brazilian pigs from 1980 to 2008; serotype 2 was the most prevalent (Coutinho et al., 2011).

Under the conditions of this study using British *Erysipelothrix* spp. isolates, the most common serotype was 2, followed by serotypes 1a and 1b. The results suggest that the majority of outbreaks should be controlled by the currently available vaccines. However, further studies are of

differences between *E. rhusiopathiae* vaccine strains and current field isolates area needed to determine if antigenic changes have evolved that may interfere with vaccine protection.

### **Conflict of interest statement**

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

### **Funding**

Funding was provided by the Biotechnology and Biological Sciences Research Council (BBSRC) Institute Strategic Programme Grant awarded to the Roslin Institute (BB/J004324/1; BBS/E/D/20241864). SAC Consulting: Veterinary Services receives funding from the Scottish Government as part of its Public Good Veterinary and Advisory Services. *Erysipelothrix* spp. isolates received from APHA were funded through the Department for Environment, Food and Rural Affairs (Defra) project ‘Scanning surveillance for Pig Diseases in England and Wales’ (ED1200).

### **Acknowledgements**

The authors wish to thank Dr Jin Cui and Dr Luigi Marongiu for assistance with the laboratory testing.

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**Table 1**

Serotypes of 128 *Erysipelothrix rhusiopathiae* isolates from pigs in the United Kingdom.

Serotype	1987-1989	1990-1999	2000-2009	2010-2015	Total
1a	1	6	7	7	21 (16.4%)
1b	1	4	6	6	17 (13.3%)
2	5	25	22	23	75 (58.6%)
5	-	2	-	-	2 (1.6%)
9	-	1	1	-	2 (1.6%)
10	-	-	2	-	2 (1.6%)
11	1	1	-	2	4 (3.1%)
15	-	-	-	1	1 (0.8%)
Untypeable	-	2	-	2	4 (3.2%)
Total	8	41	38	41	128

**Table 2**

Clinical signs, age and vaccination history of *Erysipelothrix* spp. serotypes 5, 9, 10, 11, 15 and untypeable isolates from pigs in the United Kingdom.

Sample	Year	Serotype	Clinical signs	Site of isolation	Pathology	Age	Vaccination status
1	1997	5	No information	Heart valve	Endocarditis	10 months	Not available
2	1998	5	No information	Heart valve	Endocarditis	14 weeks	Not available
3	1997	9	Sudden death	Liver	Septicaemia	3 weeks	Not available
4	2009	9	Cough, inappetence, weight loss, death	Heart valve	Endocarditis	4 months	Not available
5	2006	10	Acute malaise, death	Joint	Arthritis	5 months	Unvaccinated
6	2007	10	No information	Heart valve	Endocarditis	18 weeks	Not available
7	1987	11	Abortion	Viscera	No information	Foetus	Not available
8	1997	11	Sudden death	Heart valve	Endocarditis	Adult	Not available
9	2014	11	No information	Heart valve	Endocarditis	6 months	Not available
10	2015	11	No information	Heart valve	Endocarditis	10 weeks	Unvaccinated
11	2013	15	Sudden death	Heart valve	Endocarditis	20 weeks	Vaccinated
12	1993	Untypeable	Skin lesions	Skin lesion		Adult	Not available
13	1996	Untypeable	Sudden death	Viscera	Septicaemia	7 weeks	Not available
14	2014	Untypeable	Found dead	Heart valve	Endocarditis	5 months	Unvaccinated
15	2014	Untypeable	Chronic lameness, inappetence, death	Heart valve	Endocarditis	7 months	Vaccinated